Use of Wild *Arachis* Species/Introgression of Genes into *A. hypogaea* L.

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ABSTRACT

The use of wild *Arachis* L. in cultivar improvement programs has been considered an option for more than 50 yr. Both A. Krapovickas and W.C. Gregory, independently, made interspecific hybridizations in the 1940s. However, only three cultivars have been released as a result of interspecific hybridizations, and only one of those has a clearly identifiable genetic component from the wild species. Several breeding lines have been reported and several germplasm releases are documented from Texas, North Carolina, and ICRISAT. At least four potential options exist for transferring genes from wild *Arachis* to the cultigen: a) The hexaploid pathway consists of crossing a diploid wild species directly with *A. hypogaea*, doubling the chromosome number to the hexaploid level, then backcrossing for several generations to restore the tetraploid condition. Several options are possible in this pathway involving various crossing schemes prior to crossing a diploid hybrid with *A. hypogaea*. North Carolina and ICRISAT have had success with this pathway. b) The diploid/tetraploid pathway has been the most successful in Texas to date. This pathway involves crossing diploid species (two to several), doubling the chromosome number of the hybrid, then crossing to *A. hypogaea* and backcrossing with selection for the desired character. This pathway is most successful when both A- and B-genome species are involved. Germplasm lines and a cultivar have been released in Texas using this pathway. c) Another diploid/tetraploid pathway could be to double chromosome numbers of diploid species and cross the amphiploids directly with *A. hypogaea*. Several attempts have been made with this technique, but no germplasm releases have been reported, in large part because sterility is too great when both A and B genomes are not included in the hybrid. Many of the sections/species of wild *Arachis* are so greatly isolated from *A. hypogaea* that plant transformation will be the likely method to introduce genes into the cultigen. d) Molecular methods of "inserting" genes into peanut that have been modestly successful and include use of *Agrobacterium* spp., electroporation, and direct DNA delivery techniques such as the gene gun, whiskers, and sonication. No releases have resulted.

Keywords: Amphiploid, hybridization, interspecific, pathways.

The desire to transfer genes from wild *Arachis* species into cultivated peanut has burned brightly since the 1940s when both W.C. Gregory and A. Krapovickas first attempted to cross wild peanuts with *A. hypogaea* L. Their first attempts were unsuccessful, but with continued effort and diligence their success rate improved over the next five decades (Gregory and Gregory, 1979; Krapovickas and Gregory, 1994).

The first peanut cultivars released from interspecific hybridization were by Hammons (1970) and Simpson and Smith (1975). Hammons released cv. Spancross in 1970 from the cross *A. hypogaea* x *A. monticola* Krapov. and Rigoni, which was the same source of cv. Tamnut 74 released by Simpson and Smith. Neither of these cultivars had phenotypic characters that could be identified as derived from the wild species. In 1999, Simpson and Starr (2001) released the first root-knot nematode-resistant peanut cultivar, COAN. This new cultivar contains a gene for the pest resistance which was transferred from *A. cardenasii* Krapov. and W.C. Gregory in an intensive backcrossing program (Simpson, 1991).

Several programs have released germplasm lines which have been derived from interspecific hybridization, including Simpson *et al.* (1993) and Stalker and Beute (1993).

Possible Introgression Pathways

There are at least three possible introgression pathways to *A. hypogaea*, and they all have variations depending upon the objective of the program and the availability of germplasm (Stalker *et al.*, 1979; Simpson, 1991). Figure 1 outlines the hexaploid route where *A. hypogaea* (2*n* = 40) is hybridized with a diploid wild *Arachis* species (2*n*

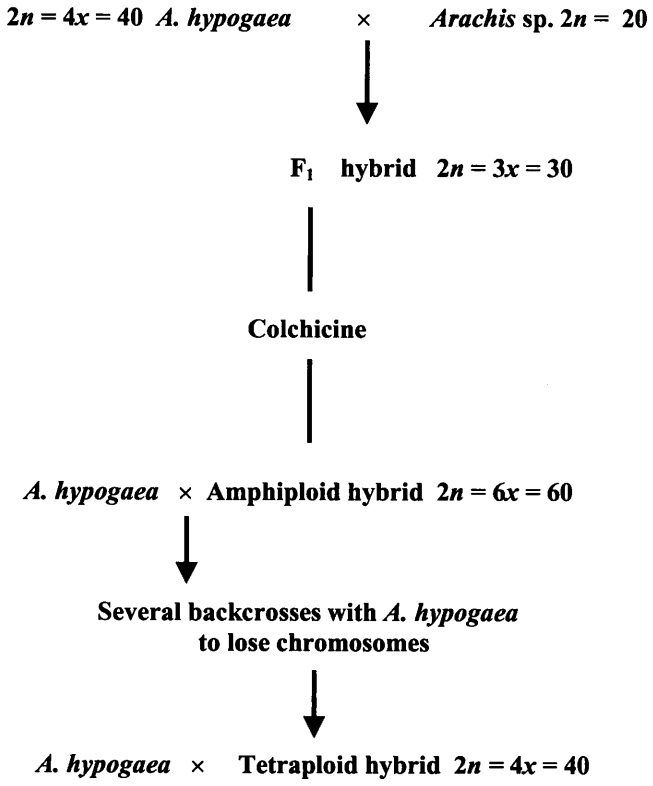

Fig. 1. Hexaploid pathway for introgression in *Arachis*.

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= 20) to produce a sterile triploid. The hybrid is then chromosome-doubled to produce a hexaploid (6x = 60). The amphiploid is first crossed and then selfed or backcrossed with *A. hypogaea* until the normal action of chromosome segregation has eliminated the excess chromosomes and a tetraploid hybrid is available for variety development. During the backcrossing cycles, selection may be practiced for the desired character(s) but sterility has a large effect on this activity. Some combinations are much easier to work with than others. For example, hexaploids produced from crosses between most virginia market-type cultivars and *A. diogoi* Hoehne or *A. cardenasii* will be highly sterile. However, if a spanish or valencia market-type cultivar is used, the hexaploid will usually be somewhat fertile. This pathway has been used with some success in North Carolina (Smartt and Gregory, 1967; Stalker *et al.*, 1979) and ICRISAT (Moss, 1985; ICRISAT, 1990). The program in North Carolina actually had a very fortuitous event at the first crossing, when they recovered a tetraploid plant from the progeny of the hexaploid (Smartt and Gregory, 1967; Stalker and Beute, 1993). The North Carolina program has released several germplasm lines (Stalker and Beute, 1993) from these progenies, but no cultivars to date. The ICRISAT program worked with derivatives of the same original population developed by Smartt and Gregory (1967) with some success. They have distributed numerous sets of disease- and insect-resistant breeding lines (Moss, 1985; Singh, 1985, 1986a,b; Moss *et al.*, 1989; ICRISAT, 1990).

There are several variations to this technique that have been or could be tried. For example, two or more diploid species could be hybridized before crossing with *A. hypogaea*, but in one such program (Simpson,1991) high levels of sterility after the first backcross resulted in the effort being abandoned.

Figure 2 outlines a diploid/tetraploid pathway that has been the most successful introgression pathway at Texas A&M for transferring genes from the wild *Arachis* species into *A. hypogaea* (Simpson, 1991; Simpson and Starr, 2001). This pathway assures inclusion of a B-genome type species in the scheme. In the Texas program, *A. cardenasii* was first crossed by *A. diogoi*. The resulting hybrid (52% pollen stained) was crossed as the male parent onto *A. batizocoi*, the "B" type genome donor. The resulting diploid three-way hybrid was sterile (pollen stained < 1%), and the plant was subsequently chromosome-doubled with colchicine. The amphiploid had above 90% pollen stained and was easily crossed with *A. hypogaea* cv. Florunner. From this point, resistant progenies that were highly fertile were selected and backcrossed to *A. hypogaea*. With this technique we have successfully transferred high levels of early (*Cercospora arachidicola* Hori) and late [*Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (syn. *Phaeolsariopsis personata* (Berk. & M.A. Curtis) Arx.)] leaf spot resistance and root-knot nematode resistance [*Meloidogyne arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood] into *A. hypogaea*. Transfer of other characters is in progress using this technique at Texas A&M Univ. (C. Simpson and J. Starr, unpubl. data).

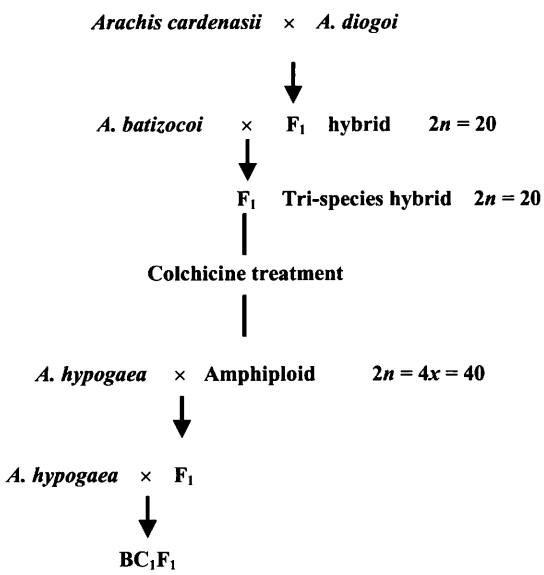

Fig. 2. Diploid/tetraploid pathway with three-way cross (used to develop cv. COAN).

Figure 3 shows a diploid/tetraploid route where two wild *Arachis* species are doubled with colchicine, then the two amphiploids are hybridized to form a tetraploid hybrid that is crossed to *A. hypogaea*, provided the amphiploid hybrid is fertile enough to make the cross. This pathway has been attempted (C. Simpson, unpubl. data), but to date has proven unsuccessful as an introgression pathway. High levels of sterility are the major factor limiting this technique.

Figure 4 shows a third diploid/tetraploid route that involves crossing two diploid wild *Arachis* species, doubling the chromosome number of the hybrid, then crossing with *A. hypogaea*. This pathway was attempted in Texas (Simpson, 1991) but, without both A and B genome types in the crossing scheme, the success is limited greatly because of high sterility factors.

Some of the sections/species of wild *Arachis* are so greatly isolated from *A. hypogaea* that biotechnology will be the only available method to introduce genes from them into *A. hypogaea*. The most distant species appear to be in sections *Trierectoides* Krapov. and W.C. Gregory, *Ambinervosae* Krapov. and W.C. Gregory, *Extranervosae* Krapov. and W.C. Gregory, and *Heteranthae* Krapov. and W.C. Gregory. Molecular

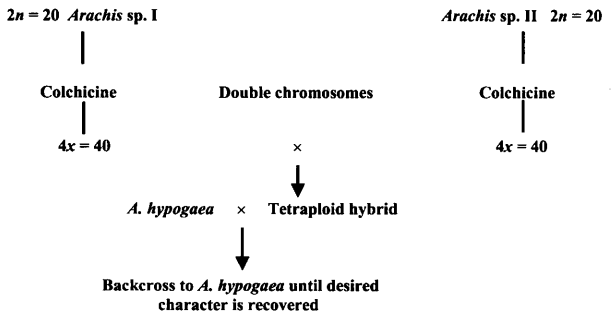

Fig. 3. Diploid/tetraploid pathway with a two-way cross.

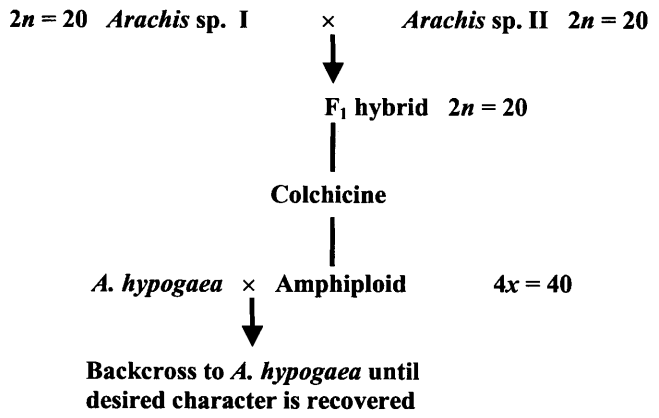

Fig. 4. Diploid/tetraploid pathway with two-way cross.

methods of "inserting" genes into peanut that have been mentioned include use of *Agrobacterium* spp., electroporation, and direct DNA delivery techniques such as the gene gun, whiskers, and sonication [see Ozias and Gill (2001) for review]. Some of these techniques are under experiment, but to date no one has been successful in producing a genetically modified (GM) cultivar of peanut.

Conclusions

The introgression of genes from the wild *Arachis* species is in its infancy, with only two or three genes for root-knot nematode resistance being transferred and three to five genes for leafspot resistance accomplished. In the future there will be a larger number of genes transferred into *A. hypogaea*. Many of these will be through conventional methods using the pathways described in this manuscript or some modification of them. The techniques of plant transformation eventually will be sufficiently refined so they also will be successful in *Arachis*. Public opinion on genetically modified organisms (GMO) likely will change as educational programs are successful, and the cultivars developed in this manner will be acceptable.

Literature Cited

Gregory, M.P., and W.C. Gregory. 1979. Exotic germ plasm of *Arachis* L. interspecific hybrids. J. Hered.70:185-193.

Hammons, R.O. 1970. Registration of Spancross peanut. Crop Sci. 10:459.

ICRISAT. 1990. ICRISAT Annual Report 1989. ICRISAT Center, Patancheru, A.P., India.

Krapovickas, A., and W.C. Gregory. 1994. Taxonomia del genero *Arachis* (*Leguminosae*). Bonplandia 8:1-186.

Moss, J. P. (ed.). 1985. Proceedings of an International Workshop on Cytogenetics of *Arachis*, Hyderabad, India, 31 Oct.–2 Nov. 1983. ICRISAT Center, Patancheru, A.P., India.

Moss, J.P., V. Ramanatha Rao, and R.W. Gibbons. 1989. Evaluating the germplasm of groundnut (*Arachis hypogaea*) and wild *Arachis* species at ICRISAT, pp. 212-234. *In* A.H.D. Brown, O.H. Frankel, D.R. Marshal, and J.T. Williams (eds.) The Use of Plant Genetic Resources. Cambridge Univ. Press, Cambridge, U.K.

Ozias-Akins, P., and R. Gill. 2001. Progress in the development of tissue culture and transformation methods applicable to the production of transgenic Peanut. Peanut Sci. 28:123-131.

Simpson, C.E. 1991. Pathways for introgression of pest resistance into *Arachis hypogaea* L. Peanut Sci. 18:22-26.

Simpson, C.E., and O.D. Smith. 1975. Registration of Tamnut 74 peanut. Crop Sci. 15;603-604.

Simpson, C.E., and J.L. Starr. 2001. Registration of COAN peanut. Crop Sci. 41:918.

Simpson, C.E., J.L. Starr, S.C. Nelson, K.E. Woodard, and O.D. Smith. 1993. Registration of TxAG-6 and TxAG-7 peanut germplasm. Crop Sci. 33:1418.

Singh, A.K. 1985. Genetic introgression from compatible *Arachis* species into groundnut, pp. 107-117. *In* J.P. Moss (ed.) Proc. Int. Workshop on Cytogenetics of *Arachis*, Hyderabad, India, 31 Oct.–2 Nov., 1983. ICRISAT Center, Patancheru, A.P., India.

Singh, A.K. 1986a. Utilization of wild relatives in the genetic improvement of *Arachis hypogaea* L. 7. Autotetraploid production and prospects in interspecific breeding. Theor. Appl. Genet. 72:164-169.

Singh, A.K. 1986b. Utilization of wild relatives in the genetic improvement of *Arachis hypogaea* L. 8. Synthetic amphidiploids and their importance in interspecific breeding. Theor. Appl. Genet. 72:433-439.

Smartt, J., and W.C. Gregory. 1967. Interspecific cross-compatibility between the cultivated peanut *Arachis hypogaea* L. and other members of the genus *Arachis*. Oléagineux 22:455-459.

Stalker, H.T., and M.K. Beute. 1993. Registration of four leafspot-resistant peanut germplasm lines. Crop Sci. 33:1117.

Stalker, H.T., J.C. Wynne, and M. Company. 1979. Variation in progenies of an *Arachis hypogaea* diploid wild species hybrid. Euphytica 28:675-684.